Free Radical Biology & Medicine, Vol. 37, No. 11, pp. 1683–1693, 2004

0891-5849/$-see front matter

ELSEVIER

doi:10.1016/j.freeradbiomed.2004.08.002

Serial Review: Flavonoids and Isoflavones (Phytoestrogens: Absorption, Metabolism, and Bioactivity)
Serial Review Editor: Catherine Rice-Evans

FLAVONOIDS AND THE BRAIN: INTERACTIONS AT THE BLOOD–BRAIN BARRIER AND THEIR PHYSIOLOGICAL EFFECTS ON THE CENTRAL NERVOUS SYSTEM

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(*Received* 16 *January* 2004; *Revised* 22 *July* 2004; *Accepted* 5 *August* 2004)
Available online 11 September 2004

Abstract—Over the past few years there has been an exponential growth in the number of reports describing the effects of nutritional modulation on aging and age-related diseases. Specific attention has been directed toward the beneficial effects afforded by dietary antioxidants, in particular those from fruit and vegetables, in ameliorating age-related deficits in brain performance. The rationale for studying the effects of dietary intervention stems from evidence implicating free radicals in aspects related to the aging process. Age-dependent neuropathology is a cumulative response to alterations induced by reactive oxygen species. Therefore cognitive aging, according to this hypothesis, should be slowed, and possibly even reversed, by appropriately increasing levels of antioxidants or decreasing overproduction of free radicals in the body. Published by Elsevier Inc.

Keywords—Flavonoids, Blood–brain barrier, Neuroprotection, Brain, Cognition, Aging, Free Radicals

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INTRODUCTION

There is considerable evidence that supports the notion that free radical generation may act as a catalyst for development of the neuropathology that underlies cognitive aging [1]. The central nervous system has a high rate of oxidative metabolism relative to other tissues, receiving almost 15% of the cardiac output and accounting for up to 30% of the resting metabolic

This article is part of a series of reviews on "Flavonoids and Isoflavones (Phytoestrogens: Absorption, Metabolism, and Bioactivity)." The full list of papers may be found on the home page of the journal.

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rate [2]. In light of this, there is no wonder it exhibits increased vulnerability with age from the cumulative effects of oxidative damage to proteins, lipids, and nucleotides. Such oxidative damage has been observed in aged brains of various mammals. It is well known, for example, that central nervous system (CNS) vulnerability to oxidative stress (OS) (see reviews [3,4]) and inflammation [5] increases during aging, and dietary antioxidant/anti-inflammatory agents may reduce these sensitivity increases.

Age-related deficits in memory occur primarily in secondary memory systems and are reflected in the retrieval of newly acquired information. The impairments in retrieval are attributed to deficits in such encoding processes as motivation, attention, processing depth and organizational skills, and have been characterized in animals [6–8] and humans [9,10]. In contrast, motor performance deficits are thought to result from alterations in the striatal dopamine system [11] or the cerebellum [12], whereas age-related memory decrements can occur from alterations in either the hippocampus (which mediates allocentric spatial navigation or place learning) or the striatum (which mediates egocentric spatial orientation or response/cue learning). Cognitive decline is also a component of specific neurological disorders including Parkinson's and Alzheimer's diseases. Youdim and co-workers, in the current series, review the beneficial effects of dietary flavonoids on the neuropathology of Parkinson's disease (PD). Our purpose is to bring the reader up-to-date on the most recent studies describing the beneficial effects afforded to brain performance as assessed in rodents, canines, and humans, with particular emphasis on changes in the optimal performance of the CNS that occur simply as a function of aging.

As described previously, a growing number of studies have found beneficial cognitive effects from counteracting oxidative stress by dietary intervention with antioxidants (see reviews [13–18]). Aged rats supplemented with vitamins E and C combined and spinach, blueberry, or strawberry extracts exhibited faster learning and better memory retention than nonsupplemented animals. Motor learning and cerebellar function also are improved in aged rats fed strawberry, blueberry, or spinach extract. In certain cases, the execution of particular tasks improved to performance levels observed in young animals. In addition, age-related changes in long-term potentiation and antioxidant defenses are reversed by foods enriched with the antioxidant α-lipoic acid [19]. Additional dietary agents that have been employed to alter behavioral and neuronal deficits with aging include herbal extracts (e.g., ginseng, ginkgo biloba, Ding lang; see [20]) and dietary fatty acids (reviewed in [21]).

Only recently have studies been performed that focus on the potential for flavonoids per se to mediate neuroprotection. It is not clear whether the neuroprotective effects of flavonoids involve their reducing properties or some other mechanism independent of their antioxidant activities. Williams and co-workers, in the current series, review some of the possible signaling mechanisms by which flavonoids may promote their neuroprotective actions. However, their precise mechanisms of action in vivo will depend on the extent to which they are conjugated and metabolized during absorption (Walle and co-workers, current series) and the ability of bioavailable compounds to localize within the brain. A lacuna in this research is that the mechanisms by which flavonoids penetrate the blood–brain barrier have not yet been addressed, an essential element if attempting to elucidate possible neuroprotective actions, active forms, and site of action.

FLAVONOID PERMEABILITY ACROSS THE BLOOD–BRAIN BARRIER

Until recently, there was little understanding of the metabolism of flavonoids and their mode of entry into the systemic circulation after oral absorption. Although numerous studies have reported flavonoid-mediated neuroprotection, there is little information about the interaction of flavonoids or their circulating metabolites with the brain endothelial cells forming the blood–brain barrier (BBB), which has complicated identification of flavonoid compounds entering the CNS. The BBB is formed by the endothelium of brain microvessels, under the inductive influence of associated cells, especially astrocytes [22]. Features that distinguish brain endothelium from that of other organs include complex tight junctions, a low density of pinocytotic vesicles, and presence of specific transporters including efflux carriers [23]. It is these properties that make the BBB a regulatory interface that selectively limits passage of most small polar molecules and macromolecules from the cerebrovascular circulation to the brain, exerting tight control over transendothelial molecular traffic, and contributes to regulation of brain extracellular fluid composition.

The flavonoid epigallocatechin gallate, a polar polyphenol predominantly found in tea and to a lesser degree in wine, has been reported to enter the brain after oral administration [24]. In addition, Peng and co-workers [25] identified both naringenin and its glucuronide in the cerebral cortex, after intravenous administration of naringenin (20 mg/kg). In a later study, Tsai and Chen [26], administered hesperetin intravenously (50 mg/kg) and, using microdialysis, detected hesperetin in the brain, especially the striatum (in contrast to Peng et al., 1998)

but no conjugates. More recently, a study has reported the presence of the epicatechin glucuronide and 3′ -*O*-methyl epicatechin glucuronide, formed after oral ingestion of epicatechin (100 mg/kg body w/day), in rat brain tissue [27]. However, most of these studies were not quantitative, and omitted or failed to report important control procedures, such as whether saline perfusion (washout) was used to reduce intravascular contamination before collecting brain tissue, or if any correction was made for residual vascular content of compounds present in whole-brain homogenates. Hence, the observed uptake may have been due to residual flavonoids in the circulation and/or in the capillary endothelium.

A recent study [28] has examined the permeability of flavonoids and their known circulating metabolites across an in vitro model of the BBB. The model employs ECV304 cells grown on filters above C6 glioma cells [29]. The ECV304 cell line is a useful model system expressing a robust endothelial phenotype and, when co-cultured with astrocytic glia, shows upregulation of a number of features characteristic of the BBB in vivo, including increased tight junctional organization and elevated TEER [30]. In this study, hesperetin, naringenin, and their respective in vivo glucuronidated conjugates, as well as the anthocyanins cyanidin-3-rutinoside and pelargonidin-3-glucoside, all showed measurable permeability across the in vitro model, correlating with calculated octanol–water partition coefficients (calculated log *P*), a measure of lipophilicity (Fig. 1). In comparison with the permeability of sucrose, (a marker of paracellular (tight junctional) transport), hesperetin and naringenin permeabilities were greater, indicating significant transcellular flux, in agreement with their high lipophilicity. By contrast the permeabilities of the anthocyanins and the glucuronide conjugates of hesperetin and naringenin were moderate, in each case lower than that measured for sucrose, suggesting some flux of these compounds via paracellular diffusion. However, the glucuronides are negatively charged molecules with molecular weights greater than that of sucrose, properties that would impede their paracellular flux; as such the measurable permeabilities observed could indicate some transcellular penetration.

While the ECV304/C6 in vitro model provides a useful screening tool for assessing and ranking passive permeability of compounds across the BBB, it, however, does not fully mimic the physiological environment, in particular the role of efflux transporters, which control xenobiotic flux across the BBB [31]. Of particular importance is the efflux transporter P-glycoprotein (P-gp), a product of the multidrug-resistant (mdr) genes, whose function at the BBB has received the greatest attention (reviewed in [31]). P-glycoprotein is an ATP-dependent efflux pump responsible for cross-resistance of human cancers to a variety of lipophilic compounds and is composed of two homologous halves, each containing six transmembrane domains and an ATP-binding/utilization domain. It is expressed on the luminal cell membrane (Fig. 2) and is able to transport (efflux) a diverse array of structurally unrelated substrates out of these cells [32]. In general, substrates are lipophilic, planar molecules and are either neutral or cationic. P-glycoprotein is also expressed at the apical membrane of

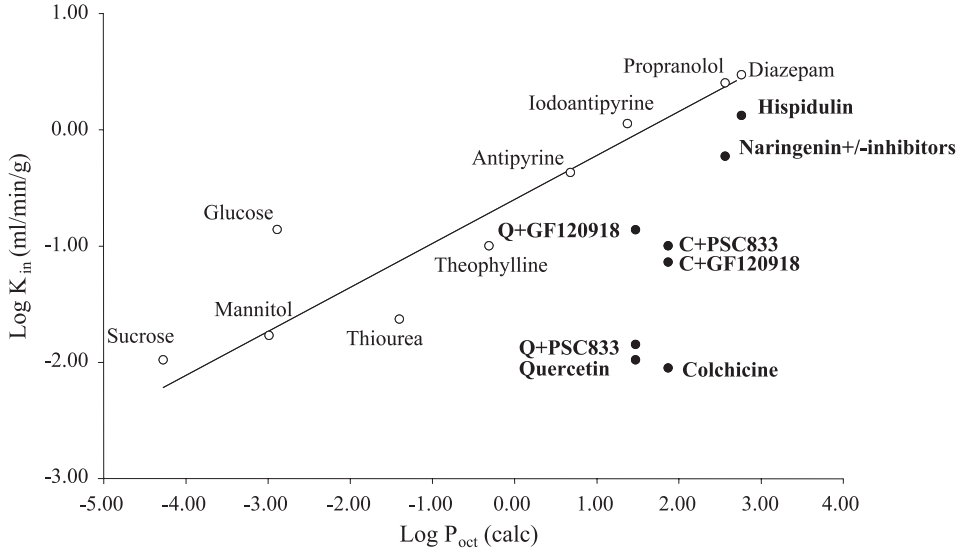

Fig. 1. Relationship between experimental log K_{in} (rate of uptake into brain) and (calculated) log P_{oct} of passively permeable compounds and efflux transport substrates. Log P_{oct} values were calculated using the KowWin (LogKow) software at http://www.esc.syrres.com. Log K_{in} values, with the exception of those for naringenin, quercetin, and hispidulin, are taken from Qaiser et al. (in preparation). Quercetin, Q; colchicine C. Adapted, with permission, from Youdim et al. [33].

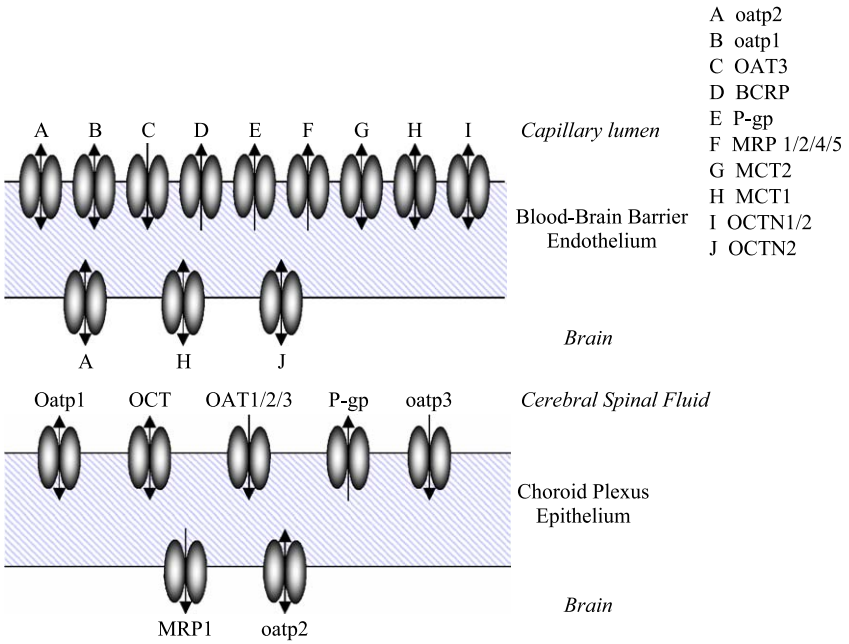

Fig. 2. Efflux transporters expressed at the blood–brain barrier (BBB) and blood–cerebrospinal fluid barrier (BCSFB). P-gp, P-glycoprotein; MRP, multidrug resistance-associated protein; BCRP, breast cancer resistance protein; MCT, monocarboxylic acid transporter; OAT, organic anion transporter; oatp, organic anion-transporting polypeptide; OCT, organic cation transporter; OCTN, organic cation transporter novel. Refer to [31] for a full description of the transporters expressed at these barriers.

the choroid plexus tissue transporting substrates into the cerebrospinal fluid (CSF) (Fig. 2). Other transporters involved in the regulation of substrates across the blood–brain interface are shown in Fig. 2; they include the multidrug resistance-associated proteins (MRPs) and the recently identified breast cancer resistance protein (BCRP) [31].

As an extension to the in vitro study describing the BBB permeability of flavonoids, we have recently elucidated the kinetics of flavonoid uptake into different brain regions, and examined whether efflux transporters at the BBB limit this [33]. These studies employed the rat in situ brain perfusion model [34], preserving additional physiological features of the in vivo BBB. Flavonoids examined were the citrus flavanone naringenin, the flavonol quercetin found in onions, and hispidulin a major polyphenolic component in sage leaves (*Salvia officinalis* L.). Naringenin and hispidulin were found to localize in the different brain regions examined, with rates of uptake (K_{in}, mL min^{-1} g^{-1}) correlating well with regional perfusate flow rates. As a positive control, colchicine, a known P-gp substrate, was employed, and also exhibited measurable flux into the different brain regions. Drugs that exhibit passive permeability across the BBB have been found to exhibit a linear relationship between Log K_{in} and calculated log P_{oct} (calculated using the KowWin (LogKow) software at http://esc.syrres.com) (Fig. 1). The rate of uptake for colchicine was less than that of passively diffusing drugs with similar lipophilicity, consistent with it being a substrate for an efflux transporter. Quercetin flux into different brain regions in situ was comparable to that of colchicine, but significantly lower than for the flavanone naringenin, which could not be explained solely by differences in lipophilicity. To examine the potential influence of the efflux transporter P-gp in limiting entry into the CNS, uptake was performed after pre-administration of P-gp inhibitor, PSC833 or GF120918 (10 mg/kg). As seen in Fig. 1, the single time point K_{in} for naringenin was not affected by pretreatment with either inhibitor, suggesting it was not an efflux transporter substrate. By contrast the K_{in} for colchicine increased after pretreatment with PSC833 and GF120918 as expected for a P-gp substrate, in agreement with previous studies [35,36], whereas that for quercetin increased only after pretreatment with GF120918. In each case the K_{in} values after pre-administration of the P-gp inhibitors were lower than that of iodoantipyrine, which has a comparable log *P*, which is likely to be the result of partial P-gp inhibition, as complete inhibition is difficult to achieve in vivo.

This study found that PSC833 was ineffective at inhibiting quercetin efflux in situ despite reports that showed GF120918 to be a more potent P-gp inhibitor. A number of hypotheses were proposed to explain this observation. The first was differential binding of PSC833 and GF120918 to the multiple binding sites identified on P-gp [37,38]. Hence quercetin and GF120918, but not

PSC833, may share the same binding site. No studies have examined the potential differential binding between the two inhibitors. It is also possible that quercetin interacts with another transporter besides/or in addition to P-gp. There is evidence that GF120918 (but not PSC833) also inhibits a new member of the ABC superfamily of membrane transport proteins: BCRP. So far there is little information on expression of BCRP (also referred to as ABCG2) in brain tissue [39,40]. Expression of BCRP or a similar efflux transporter, for which quercetin may be a substrate, could explain the inhibitory effect of GF120918 and lack of effect of PSC833. However, numerous studies have reported interactions between flavonoids and P-gp (Table 1), and it is therefore more likely that P-gp modulates the flux of certain flavonoids, e.g., quercetin, across the BBB, rather than BCRP. However, the role of BCRP cannot be discounted and further characterization of the functional expression of BCRP at the rat BBB is required to corroborate the role of BCRP.

INTERACTIONS BETWEEN FLAVONOIDS AND P-GLYCOPROTEIN

As described previously, the expression and role of P-gp at the BBB have received the greatest attention to date. Despite data highlighting the role played by P-gp in limiting drug entry into the CNS, there is no direct information on its role in limiting flavonoid entry. There are only indirect studies highlighting interactions between P-gp and flavonoids that one could possibly

Table 1. Various Studies Reporting Interactions between Flavonoids and P-Glycoprotein

Flavonoid	P-gp expressing cell line used	Substrate	Effective concentration (μM)	Effect on P-gp-mediated substrate efflux	Ref.
Quercetin	HCT-15 colon cells	Adriamycin		Stimulation	[52]
	MBEC4	Vincristine	10/50	Stimulation/inhibition (biphasic concentration-dependent effect)	[53]
	Fresh rat hepatocytes (72 h-cultured)	Rhodamine 123/doxorubicin	100	Inhibition/stimulation (substrate-dependent)	[54]
Galangin	HCT-15 colon cells	Adriamycin		Stimulation	[52]
	Fresh rat hepatocytes (72 h-cultured)	Rhodamine 123/doxorubicin	100	Inhibition/stimulation (substrate-dependent)	[54]
Kaempferol	HCT-15 colon cells	Adriamycin		Stimulation	[52]
	MBEC4	Vincristine	10/50	Stimulation/inhibition (biphasic concentration-dependent effect)	[53]
	Fresh rat hepatocytes (72 h-cultured)	Rhodamine 123/doxorubicin	100	Inhibition/stimulation (substrate-dependent)	[54]
Chrysin	MBEC4	Vincristine	10–50	Dose-dependent inhibition	[53]
Hesperetin	MBEC4	Vincristine	10–50	Dose-dependent inhibition	[53]
Naringenin	MBEC4	Vincristine	10–50	Dose-dependent inhibition	[53]
Rosemary extract	MCF-7	Doxorubicin and vinblastine	50	Inhibition	[55]
Orange juice (50% ethyl acetate extract)	Caco-2	Vinblastine	50:50 (v/v) in water	Inhibition	[56]
3,3′ ,4′ 5,6,7,8-Heptamethoxyflavone	Caco-2	Vinblastine	5–100	Dose-dependent inhibition	[56]
Tangeretin	Caco-2	Vinblastine	5–100	Dose-dependent inhibition	[56]
Biochanin A	MCF-7	Daunomycin	5–50	Dose-dependent inhibition	[57]
	Caco-2	Digoxin and vinblastine	5–100	Dose-dependent inhibition	[58]
Morin	MCF-7	Daunomycin	5–100	Dose-dependent inhibition	[57]
Phloretin	MCF-7	Daunomycin	5–100	Dose-dependent inhibition	[57]
Silymarin	MCF-7	Daunomycin	5–100	Dose-dependent inhibition	[57]
	Caco-2	Digoxin and vinblastine	5–50	Dose-dependent inhibition	[58]
Epigallocatechin	CH^RC5	Rhodamine 123	100	Inhibition	[59]
	NIH3T3-G185	LDS-751	50–250	Dose-dependent inhibition	[60]
Catechin gallate	CH^RC5	Rhodamine 123	100	Inhibition	[59]
Epigallocatechin gallate	CH^RC5	Rhodamine 123	100	Inhibition	[59]
	NIH3T3-G185	Rhodamine 123	50–250	Dose-dependent inhibition	[60]
Resveratrol	CH^RC5	Rhodamine 123	100	Inhibition	[59]
St. John's wort	LS-180V	Rhodamine 123	300–100 μg/ml	Stimulation	[61]
Epicatechin gallate	NIH3T3-G185	Rhodamine 123 and LDS-751	50–250	Dose-dependent inhibition	[60]
Epicatechin	NIH3T3-G185	LDS-751	50–250	Dose-dependent stimulation	[60]
Catechin	NIH3T3-G185	LDS-751	50–250	Dose-dependent stimulation	[60]

extrapolate to the physiological scenario at the BBB. Table 1 highlights the flavonoids that have been shown to interact with P-gp. The exact mechanism(s) by which flavonoids modulate P-gp efflux is still unclear. For example, they could bring about changes in the physicochemical properties of the drug recognition pockets in the protein without changing conformation of the transporter. There is also the possibility that flavonoids do not interact with P-gp directly but modulate its function by altering plasma membrane properties or factors contributing to the activity of the transporter. In light of the growing evidence suggesting interactions between flavonoids and the steroid-interacting regions and ATP binding sites within the P-gp nucleotide binding domains, the latter hypothesis would appear less feasible. Hence differential binding/interactions of different flavonoids with these sites may explain the potency of certain compounds and the lack of effect with others. The interactions between flavonoids and P-gp (which could potentially limit their entry into the CNS) have recently been reviewed in detail and as such are not covered here.

NEUROPROTECTIVE ACTIONS OF FLAVONOIDS

Protection afforded to the blood–brain barrier

We have previously discussed the ability of flavonoids to cross the BBB as observed in vitro and in situ. The primary route appears to be via transcellular diffusion, and depends on the properties of the flavonoids, such as charged state and lipophilicity. In crossing the BBB, one might expect retention of flavonoids within the capillary endothelium. In this regard the first line of neuroprotection may in fact reside at the blood–brain interface. Despite few studies having directly shown the permeability of flavonoids across the BBB, there are studies that report cellular retention of flavonoids. Uptake of anthocyanins by aortic endothelial cells [41] has previously been shown to improve their viability against oxidative damage. Protection afforded to endothelial cells against reactive oxygen and nitrogen species [42–45] and inflammatory insults [46–48] by dietary forms of flavonoids has also been reported. This may have important implications, as oxidative stress may contribute to age-related breakdown of BBB function and the related neuronal dysfunction [49]. However, these observations are made using endothelial cells that are not derived from the brain microvasculature and, as such, may not express important efflux transporters such as those known to function at the BBB. In light of the reports that describe interactions between flavonoids and P-gp, the potential protective effects of these compounds at the BBB require further examination. However, uptake of flavonoids into rat brain endothelial cells has recently been reported [28], although their protective effects were not investigated. More direct evidence supporting the beneficial effects of flavonoids at the BBB comes from studies showing that anthocyanin supplementation of rats protected the BBB against collagenase-induced increases in permeability [50]. More recently procyanidin oligomers were found to increase the resistance of rat brain capillaries to breakdown after intraventricular administration of bacterial collagenase [51].

Beyond the antioxidant/Anti-inflammatory effects

If, as pointed out above, the BBB is permeable to certain flavonoids, then one might question whether their regional localization after dietary administration may relate to behavioral performance. Indeed, LC MS/MS analyses of anthocyanins in several brain regions obtained from senescent animals supplemented with blueberries for 8 weeks showed that these flavonoids (e.g., malvidin glucoside) were localized in regions such as the cortex, hippocampus, cerebellum, and striatum. Interestingly, it appeared that the number of different anthocyanins (not their total amounts) in the cortex were negatively correlated with rats' latency to find the platform on Day 4 in the Morris water maze ($r = -0.78$) (Andres-Lacuava et al., in preparation). The Morris water maze is the most popular task in behavioral neuroscience, assessing spatial learning and memory. Performance in the Morris water maze is acutely sensitive to manipulations of the hippocampus. This relationship is being assessed with respect to other areas of the brain, but given these findings it might be surmised that the beneficial properties of fruit such as berryfruit might involve more than simply enhancing antioxidant/anti-inflammatory activities.

Evidence from several articles suggests that to varying degrees flavonoids may have a direct effect on cell signaling [62–65]. Additionally, Joseph et al. [66] evaluated the effects of blueberry supplementation on the behavioral deficits and amyloid pathology that develops in an APP + PS1 transgenic mouse model of amyloid deposition [67–72]. The results of the Joseph et al. [66] study showed that the blueberry diet protected the mice from developing the deficits in memory in the Y maze without altering amyloid β (Aβ) loads in hippocampus and frontal cortex. The Y maze is used to assess (without food deprivation or other aversive procedures) the normal navigation behaviors of rodents. Test subjects are placed in a Y-shaped maze for 5 to 8 min. All arm entries are sequentially scored so that the total number of arm entries, as well as the sequence of entries, is recorded. Data are analyzed to determine the number of arm entries without repetition. Success in this test is indicated by a high rate of alternation in the control groups, indicating that the animals can remember which

arm was entered. This test has been shown to be sensitive to hippocampal damage and gene manipulations. These data suggested that it is possible for blueberry supplementation to alter behavior in the transgenic mouse without affecting the pathology.

Further analysis revealed that the preservation of behavioral performance in blueberry-supplemented transgenic animals may have involved the enhancement of several neuronal signals that are important in learning and memory (Fig. 3) [66]. These include muscarinic receptor GTPase activity, hippocampal protein kinase α (PKCα), and extracellular signal-regulated kinase (ERK). The changes in striatal carbachol-stimulated GTPase activity were significantly correlated with Y-maze performance, while there was a trend toward a significant correlation with phospo-PKC. Thus, because some of these indices were also correlated with Y-maze performance, it is plausible that blueberry supplementation facilitates behavioral performance by enhancing neuronal signaling. Multiple studies have shown that PKC activity is important in memory formation, particularly spatial memory (reviewed in [73]), and that treatment with PKC inhibitors impairs memory formation [74]. PKC may also interact with several signaling molecules that are described below, and is involved in a great amount of cross-talk with other signaling molecules such as protein kinase A and PTK in the initiation of memory formation and the conversion of short- to long-term memory (Fig. 3).

In the case of ERK, a great deal of work has shown the importance of the MAP kinase (MAPK) cascade in proliferation and differentiation [75]. However, evidence is increasing that MAPK is critical in long-term memory formation through the activation of calcium response element-binding protein (CREB) (Fig. 3), which is involved in the formation of memory and regulates the transcription of immediate early genes (reviewed in [76,77]). Lamprecht [76], for example, cites research that CREB affects the growth of new synapses and synaptic transmission, and Bourtchuladze and co-authors [78] have shown that CREB knockout mice were impaired in Morris water maze performance. CREB activity also has been shown to decline with age [79]. Conversely, Josselyn and co-authors [80] have shown that overexpression of CREB enhances the formation of long-term memory after massed training. More specifically, there are a great amount of data indicating that MAPKs are involved not only in hippocampal memory formation but also in memory modulation in other brain structures [81]. Moreover, recent studies have indicated that the activation of these molecules is sensitive to oxidative stress [82] and they may serve as biochemical signal integrators and/or molecular coincidence detectors for modulating coordinated responses to extracellular signals in neurons [83]. Particularly important in this regard are ERK1 and ERK2. Studies have demonstrated the role of ERK signaling cascades in diverse types of learning and memory such as conditioned taste aversion [84], novel taste learning [85], spatial learning [86], and inhibitory avoidance [87].

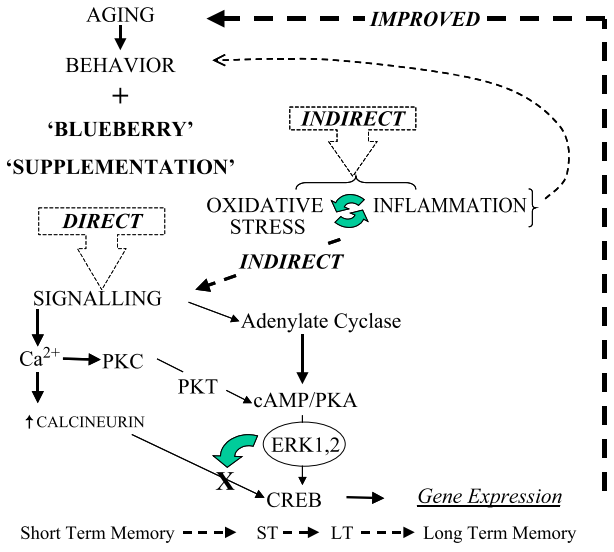

Fig. 3. Possible direct and indirect effects of blueberry supplementation on learning/memory-induced signaling cascades.

Also important is the finding that ERK activities were reduced in cortical brain slices of senescent rats (24 months) without declines in the corresponding protein levels [88]. An additional study showed that exposure of hippocampal slices from senescent mice expressing $A\beta_{1-42}$ produced downregulation of hippocampal ERK activity [89]. Finally, preliminary findings from the authors' laboratory indicate that these alterations in ERK activity in response to oxidative stress are area- and age-specific. Given their critical roles in memory, especially the conversion of short- to long-term memory, the above findings in transgenic animals suggest that the investigation of these molecules may be important in determining the putative sites of action and mechanisms involved in the beneficial effects of dietary supplementation with flavonoids such as those found in blueberries.

A second possible effect of blueberry supplementation that may also reflect more than just antioxidant and anti-inflammatory properties is that such supplementations may actually increase neurogenesis. While neurons and glia of the mature CNS are believed to be generated primarily during early postnatal life, it is now known that the brain exhibits much more plasticity than was once thought. Formation of new neurons in different areas of the brain, such as the hippocampus [90], the olfactory bulb [91], and certain areas of the cortex [92], has been shown to continue throughout life. Nevertheless, despite the

continuous proliferation of new cells in the CNS, neurogenesis decreases with age [93].

During the past several years, studies have identified various factors that can upregulate [94–102] or downregulate [93,103,104] the production and survival of hippocampal neurons developed during adulthood. In particular, it has been shown that an enriched rearing environment [95,96,100] can alter the rate of neurogenesis decline seen during aging, possibly by enhancing the survival rate of these cells [92,102,105].

Additionally it appears that newly generated neurons in the hippocampus modulate some types of hippocampus-dependent memory [106]. As mentioned above, because we have shown that age-related decrements in cognitive function (as measured by Morris water maze performance) can be reversed via blueberry supplementation (see [18]), it may be that one mechanism involved in improved memory function is the blueberry-induced increase in neurogenesis. In this regard, preliminary data depicted in Table 2 show that neurogenesis (using field counts) may be increased in the blueberry-supplemented animals. Importantly, when radial arm water maze (RAWM) performance and neurogenesis were assessed in blueberry-supplemented or control rats, animals receiving the blueberry-supplemented diet made fewer total, reference, and working memory errors than non-supplemented controls. Moreover, the number of reference and working memory errors correlated significantly with the degree of neurogenesis in the dentate gyrus (Table 2) (Casadesus et al., in preparation).

Although the results concerning signaling and neurogenesis are observed in studies using transgenic mice and old rats, respectively, taken together these findings suggest that blueberry supplementation may actually enhance neuronal communication, even in the face of possible deficits induced by pathogenesis and aging. Additionally, as has been suggested by Kempermann et al. [107], as the "gateway to memory" any small increase in neurons in the hippocampus (dentate gyrus) might increase random access memory (RAM), wherein a small number of neurons can have a profound influence on cognitive function. These possibilities are being explored; however, it is becoming clear that flavonoids may have a multiplicity of direct and indirect effects that can profoundly affect a variety of neuronal parameters that lead to alterations in motor and cognitive behavior.

Table 2. Correlation between "Neurogenesis" and Radial Arm Water Maze Performance on Day 4 in Senescent Blueberry Supplemented and Control Senescent Rats[a]

	r	*p*
Total errors (any wrong arm entry)	−0.58	<.07
Reference memory errors (enters an arm that does not contain the platform)	−0.65	<.04
Working memory errors (revisits an arm)	−0.65	<.04

[a] Rats given 5 trials a day and the platform moved on Day 4. There are no probe trials.

CONCLUSION

The aim of the current review was to bring the reader up-to-date on the most recent findings with respect to the beneficial effects of dietary flavonoids on CNS performance, with particular emphasis on cognitive performance and memory performance, which are known to decline as a function of aging but are also components of disorders such as AD. Despite increased awareness of the chemical forms in which flavonoids are able to enter the circulation, there are still a large proportion of studies that examine the underlying neuroprotective actions of flavonoids using non-physiologically relevant forms. Moreover, only recently have studies begun to consider if those flavonoids are able to enter the CNS by crossing the BBB: *because it is in the blood, does not mean it is in the brain*. In this regard, we have recently found that flavonoids from particular families are able to permeate the BBB, whereas the entry of others is limited by the actions of efflux transporters expressed at the endothelium surface. However, it should be noted that penetration of the BBB does not necessarily equate to entry into neurons, where flavonoids are believed to elicit their neuroprotective effects. Further neuropharmacokinetic data are clearly required to understand the effect of interstitial fluid (ISF) flow. Brain ISF flow is convective, induced by continual secretion at the endothelium of the BBB, and flows slowly along a resistance pathway through the extracellular space of the brain parenchyma, entering the ventricular CSF across the ependymal lining of the ventricles or the subarachnoid CSF across the pia [31]. The resistance to flow differs in different brain regions (ISF flows via easiest route). Hence, the brain regions most likely to be affected by flavonoids are those most accessible. Furthermore, the constant turnover of brain extracellular fluid, approximately every 20 h, constitutes a major efflux pathway from the brain for the contained flavonoids. With more detailed neuroparmacokinetic profiles of dietary flavonoids we can begin to correlate their localization with their recently reported effects on amyloid pathology, regulation of cell signaling pathways, and neurogenesis. With this information we can then begin to determine if their effects in specific brain regions are afforded directly or indirectly.

Acknowledgments—K.A.Y. gratefully acknowledges financial support from the Wellcome Trust (Grant 067018) and also the use of PSC833 (Novartis) and GF120918 (G.S.K.), which were gifts to Dr. David Begley, King's College London.

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